PRODUCTION AND MOLECULAR CYTOGENETIC IDENTIFICATION OF NEW WINTER WHEAT/WINTER BARLEY DISOMIC ADDITION LINES

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Abstract: Disomic wheat/barley addition lines were developed from hybrids produced with the German two-rowed winter barley cultivar 'Igri' and the Ukrainan six-rowed winter barley cultivar 'Manas'. The 2H, 3H and 4H disomic addition lines of winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' produced in Martonvásár were identified using GISH, FISH and SSR markers. A disomic addition of the 1HS isochromosome was also identified. The 2H, 3H and 4H addition lines have been multiplied in the nursery and characterized morphologically. Backcross progenies were produced and identified with molecular genetic and cytogenetic methods from the Japanese wheat 'Asakaze komugi' × Ukrainan six-rowed winter barley 'Manas' hybrids. A deletion and a translocated chromosome facilitated the physical localization of microsatellite markers on chromosome 5H. So far the 4H disomic addition line has been identified from the fertile BC_2 plants in this combination, but the development of other disomic additions is in progress

Keywords: disomic addition lines, wheat-barley addition lines, molecular cytogenetic identification

INTRODUCTION

The hybridization of wheat and barley makes it possible to transfer useful characters such as earliness, tolerance to drought and soil salinity, and various nutrition quality parameters from barley into wheat. The first wheat-barley hybrid was produced by Kruse (1973) and not much later a set of wheat/barley addition lines was developed by Islam et al. (1978). Since the production of the Chinese Spring/Betzes spring wheat/spring barley addition lines there has been no report on the development of a new set of wheat/barley addition lines except the 5H and 6H addition lines produced

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 707–713.

from a hybrid involving the wheat cultivar 'Shinchunaga' and the barley cultivar 'Nyugoruden' by Koba and co-workers in 1997. However, wheat/rye addition lines have been developed in many cultivar combinations (Shepherd and Islam, 1988). Wheat-alien addition lines form the starting point for producing translocations from selected chromosomes and are also suitable genetic materials for genome mapping. As there is great genetic variability between various barley cultivars for important agronomic traits (two or six-row, winter or spring habit, biotic and abiotic resistance, etc.) it would be advisable to develop addition lines using different barley genotypes in order to map and transfer favourable agronomical characters from barley. Many cultivated barley varieties were used for the pollination of wheat, and the development of addition lines was started from hybrids produced with the German two-rowed winter barley cultivar 'Igri' and the Ukrainan six-rowed winter barley cultivar 'Manas'.

MATERIAL AND METHODS

Plant Materials

Wheat × barley hybrids were produced using two wheat (*Triticum aestivum* L.) genotypes as the maternal plants: the Hungarian winter wheat line Martonvásári 9 kr1 (Mv9 kr1), (Molnár-Láng et al. 1996), and the Japanese wheat variety Asakazekomugi. The winter barley (*Hordeum vulgare* L.) cultivars used as the male parent were the following: Igri, (two-rowed, German) and Manas (six-rowed, Ukrainian).

The wheat × barley hybrids ('Mv9 kr1' × 'Igri', 'Asakaze komugi' × 'Manas') were multiplied in tissue culture as described earlier (Molnár-Láng et al. 2000). The backcross pollination was carried out using the wheat genotypes 'Mv9 kr1', 'Asakaze komugi' and 'Chinese Spring'. One day after pollination 100 ppm 2,4-D was injected into the stem, and embryo culture was initiated three weeks after pollination.

Cytology, sequential genomic and fluorescence in situ hybridization: Mitotic and meiotic chromosome counts were made using the Feulgen squash technique. Fluorescence in situ hybridization (FISH) was carried out with the GAA satellite sequences amplified from barley (Pedersen et al. 1996), pAs1 isolated from Ae. tauschii (Rayburn and Gill, 1986), HvT01 subtelomeric tandem repeat amplified from barley (Schubert et al. 1998) and pTa71 isolated from wheat (Gerlach and Bedbrook 1979). The DNA probes were labelled by PCR (Vrána et al. 2000) and by nick translation using either Fluorogreen (fluorescein-12-dUTP, Roche) or Fluorored (rhodamine-5-dUTP, Roche). FISH was carried out according to Linc et al. (1999). After rinsing the preparations, genomic in situ hybridization (GISH) was carried out on the same slides as described earlier (Molnár-Láng et al. 2000) following the method of Reader et al. (1994). Total barley genomic DNA was labelled with Fluorored by nick translation and used as a probe. Unlabelled wheat genomic DNA was sheared by autoclaving and used as blocking DNA at 40

times the quantity of the probe. The slides were counterstained with DAPI (4′,6-diamidino-2-phenylindole, Amersham). The chromosomes were examined under a Zeiss Axioskop 2 epifluorescence microscope equipped with Filter 10 for FITC, Filter 15 for Texas Red and Filter 01 for DAPI. The images were captured by means of a Spot CCD camera (Diagnostic Instruments, USA). The images obtained during FISH with different probes were merged using the computer program Image-Pro Plus 4.0 (Media Cybernetics, USA).

SSR marker analysis: Genomic DNA was isolated from the 'Mv9 kr1'/'Igri' disomic addition lines and the BC2 progenies of the 'Asakaze komugi' × 'Manas' hybrids and their progenitors. Altogether twenty-three SSR markers were selected from a highly saturated genetic map of barley (Ramsay et al. 2000). The markers were: Bmac0213 (1HS), Bmac0032 (1HS), EBmac0783 (1HL), HVHVA1 (1HL), HVM36 (2HS), Bmac0093 (2HS), EBmac0415 (2HL), HVM60 (3HL), HVM62 (3HL), HvLTPPB (3HS), Bmag0013 (3HL), HVM40 (4HS), HVM67 (4HL), Bmac0306 (5HS), Bmag0337 (5HL), Bmag0394 (5HL), Bmag0323 (5HL), Bmac0096 (5HL), EBmac0824 (5HL), Bmac0316 (6HS), Bmac0040 (6HL), EBmac0806 (6HL) and Bmag0120 (7HL). The PCRs were performed as described earlier (Molnár-Láng et al. 2005) following the method of Ramsay et al. (2000).

RESULTS AND DISCUSSION

Production of New Winter Wheat 'Martonvásári 9 kr1'/Winter Barley 'Igri' Disomic Addition Lines

The 'Martonvásári 9 kr1' × 'Igri' hybrid was vigorous and had good tillering ability, but showed complete male and female sterility, so it was multiplied in tissue culture from young inflorescences (Molnár-Láng et al. 2000). 101 plants were regenerated from one initial hybrid plant, of which 92 were raised to maturity. Six BC_1 plants were grown from the nine embryos developed after pollinating 4606 flowers on the regenerated 'Mv9 kr1' × 'Igri' hybrids with the wheat line 'Mv9 kr1'. Plants with 44 chromosomes were selected from the selfed progenies of the BC_2 plants. Chromosome pairing was analysed in metaphase I of meiosis to identify plants with 22 bivalents. As several different lines with 44 chromosomes and 22 bivalents in meiosis were selected, the next step was to identify the barley chromosomes added to the wheat background. At first the parental genotypes had to be identified under our laboratory conditions using FISH with the help of different DNA probes.

Identification of the Barley Chromosomes in the 'Mv9 kr1'/'Igri' Disomic Addition Lines Using FISH and SSR Markers

Wheat chromosomes can be identified using FISH with the GAA sequence combined with the pAs1 clone (Pedersen and Langridge 1997). Barley chromosomes can also be identified using FISH with the help of the GAA sequences

(Pedersen et al. 1996). The FISH hybridization pattern of barley chromosomes with the HvT01 tandem telomeric repeat and the pTa71 probe is described by Schubert et al. (1998) and by Leitch and Heslop-Harrison (1992). All the chromosomes of the parental genotypes, the wheat line 'Mv9 kr1' and the barley cultivar 'Igri' were identified with the help of these sequences in the molecular cytogenetic laboratory in Martonvásár.

The first addition line was identified using FISH with the help of the GAA sequence and the pAs1 probe. All the wheat chromosomes could be identified with the help of these sequences and a pair of 4H chromosomes was present. The 4H chromosome could be identified by its strong GAA FISH hybridization signals. The presence of the additional barley chromosome pair was confirmed with GISH. The GISH experiment was carried out on the same slides after washing off the FISH signals. Chromosome 4H was also identified with the help of probe HvT01, which gave telomeric signals on both chromosome arms, while the wheat chromosomes gave no signals. The presence of chromosome 4H in this addition line was also confirmed with SSR markers. Markers HvM 40 and HvM 67, previously mapped on chromosome 4H, gave the expected bands on this line. The 4H Mv9 kr1/Igri addition line has a compact spike with small awn stubs at the top. The spikes have good fertility. The 4H addition line has already been multiplied in our nursery, so a great number of seeds are available for further studies.

The next addition line contains a pair of 2H chromosomes, identified with a combination of the probes GAA and pAs1 using FISH. The GAA FISH hybridization signals on chromosome 2H are very similar to the C-banding pattern of this chromosome, having symmetrical interstitial hybridization sites on both arms (Jensen and Linde-Laursen 1992, Linc and Molnár-Láng 2003). The presence of 2H was also confirmed with sequential GISH analysis after FISH. When applying HvT01 as a probe, a FISH hybridization signal was observed only on the short arm of the barley chromosomes, which is typical of 2H. The other barley chromosome which has a signal only on the short arm is 5H, but that is a satellited chromosome which could be easily recognized. The SSR marker HvM36 also gave the expected PCR product size to confirm the presence of 2H in this line. This line has a long, loose spike, having fewer seeds/spike than the 4H addition line. The plants are taller than the 4H addition line. This line has also been multiplied in the nursery so a large number of seeds are available for further studies.

The third addition line contains the chromosome 3H. It was first identified using FISH with a combination of the DNA probes GAA and pAs1. The GAA hybridization sites on this chromosome did not allow the unequivocal identification of 3H, as it was mostly hybridization sites close to the centromere that could be recognized, which are very similar to the hybridization signals on 7H. Thus, the probe HvT01 was also used for FISH identification. This probe made it clear that this chromosome was 3H, as it is the only barley chromosome which has a strong subtelomeric signal and a weak interstitial signal besides the telomeric

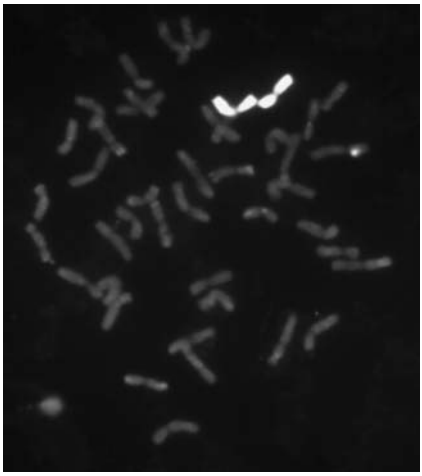

Figure 1. Genomic *in situ* hybridization on the metaphase chromosome complement of a 3H 'Mv9 kr1'/'Igri' disomic addition line. Total barley genomic DNA was labelled with Fluorored (two barley chromosomes are bright)

signal on its long arm (Schubert et al. 1998) (Fig. 1). The molecular cytogenetic identification was also confirmed with the help of molecular markers. The SSR markers HvM60 and HvM62 gave the expected bands, confirming the presence of the 3H chromosome in this line. The 3H addition line has a very compact ear with a great number of spikelets per ear. Small awnstubs can be seen at the top of the spikes. The plants are short but have good fertility. This addition line has also been multiplied in the nursery.

The fourth line was the most difficult to identify as it has a disomic addition of the isochromosome 1HS. In this addition line strong GAA FISH hybridization signals were observed around the centromere on the barley chromosome after the identification of all the chromosomes with the GAA sequence and the HvT01 repeat. It was thought to be 7H, as two telomeric bands were observed with the HvT01 probes at the ends of the chromosome pair. 5H and 6H could be excluded, as there was no satellite, and 2H and 4H on the basis of the GAA pattern, while 3H could be excluded as it has a very characteristic hybridization pattern with HvT01. However, molecular markers tested to confirm the presence of 7H did not give the expected PCR products. FISH hybridization with the probe pTa71 identified this chromosome pair as 1HS isochromosomes. Two minor, perfectly symmetrical, interstitial pTa71 signals were observed on both arms of this chromosome. The strength of this signal coincided with the strength of the signal on the 1H short arm. It thus became clear that this line carries a pair of 1HS isochromosomes. It has a long, loose spike with small awnstubs. The plant was grown in a controlled environment, producing enough seeds for further multiplication.

Production and Molecular Cytogenetic Identification of Backcross Progenies from the Wheat 'Asakaze Komugi' × Winter Barley 'Manas' Hybrids

The aim of this study was to produce backcross progenies on a new wheat ('Asakaze komugi') × Ukrainan six-rowed winter barley ('Manas') hybrid produced in Martonvásár. As no backcross seeds were obtained on the initial hybrids, young inflorescences of the hybrids were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle in metaphase I of meiosis (Molnár-Láng et al. 2005). The seven barley chromosomes were present even after the third in vitro multiplication cycle but abnormalities were observed. Sixteen BC2 plants shown by GISH analysis to contain one to three complete barley chromosomes, two deletion barley chromosomes and a dicentric wheat-barley translocation were grown to maturity from the single backcross progeny. The barley chromatin was identified using 20 chromosome-specific barley SSR markers. All seven barley chromosomes were represented in the BC2 plants. A deletion breakpoint at FL ±0.3 on the 5HL chromosome arm facilitated the physical localization of microsatellite markers. The chromosome constitution of each BC2 plant was determined with a combination of GISH and SSR markers, giving an exact demonstration of the number and origin of the barley chromosomes present in these plants. The presence of two barley chromosomes in a wheat background was detected by GISH from selfed BC2 seeds originating from a 4H monosomic addition, so three plants with 4H disomic additions were selected from the selfed seeds of the BC2 progenies. The development of the other disomic addition lines is in progress.

ACKNOWLEDGEMENTS

The technical assistance of Mrs I. Bucsi and Mrs. J. Havasi is gratefully acknowledged. This work was financed by the Hungarian National Scientific Research Fund (No. T 043502).

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